

AD-A271 891



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12 April 1993

Reprint

PEB1, the Major Cell-binding Factor of Campylobacter Jejuni, Is a Homolog of the Binding Component in Gram-negative Nutrient Transport Systems

Army Project Order
90PP0820

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Research Service
1310 24th Avenue South
Nashville, Tennessee 37212-2637

93-26166

U.S. Army Medical Research & Development Command
Fort Detrick
Frederick, Maryland 21702-5012

Title of Project Order: Studies of the Outer Membrane Proteins of Campylobacter Jejuni for Vaccine Development

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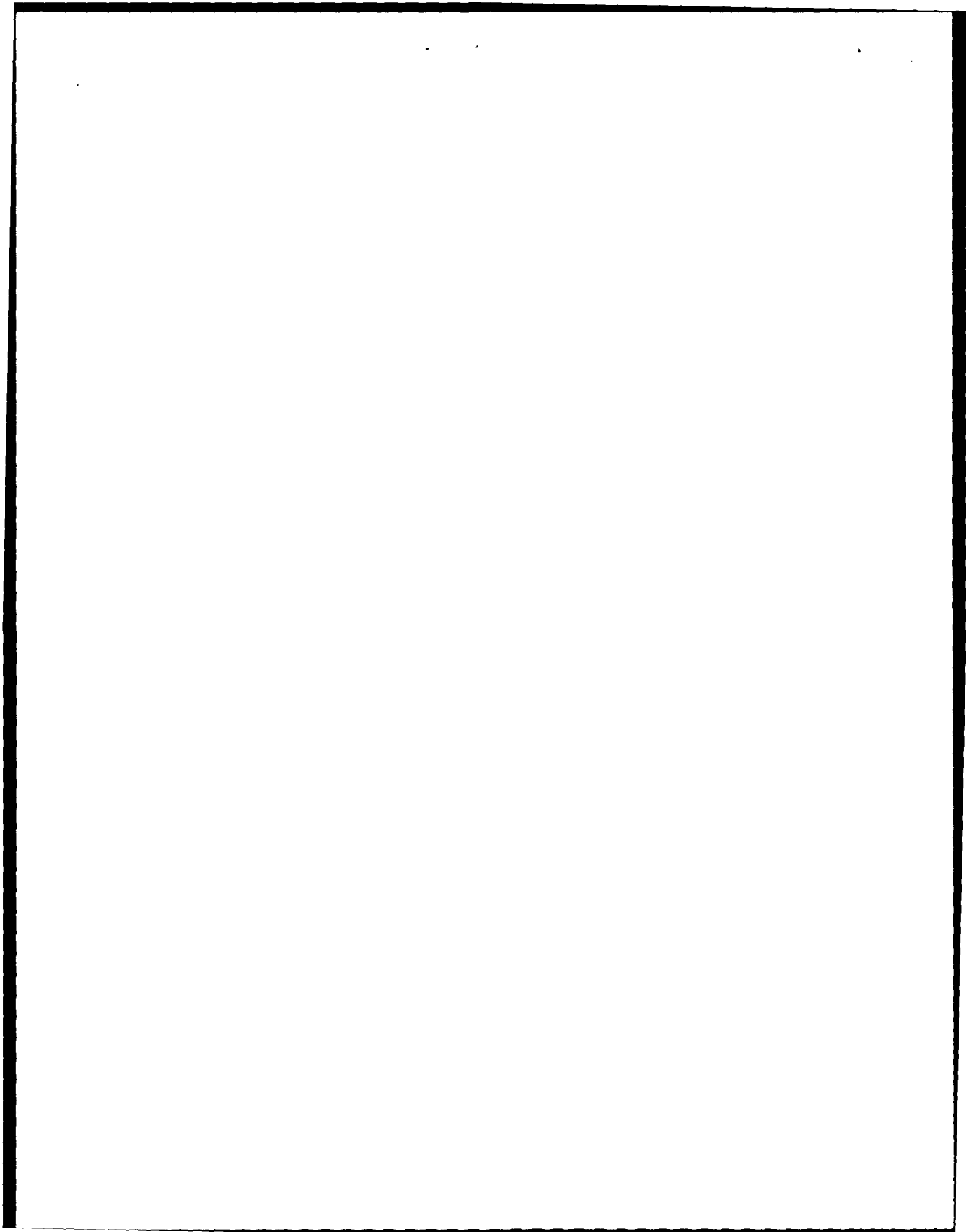
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PEB1, the Major Cell-binding Factor of *Campylobacter jejuni*, Is a Homolog of the Binding Component in Gram-negative Nutrient Transport Systems*

(Received for publication, February 12, 1993, and in revised form, April 12, 1993)

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The protein PEB1 (28 kDa) is a common antigen and a major cell adherence molecule of *Campylobacter jejuni* and *Campylobacter coli*. We created a bank of chromosomal DNA fragments of *C. jejuni* strain 81-176 using λ gt11. Screening this bank in *Escherichia coli* Y1090 cells with antibody raised against purified PEB1 enabled us to isolate and to purify a clone with a 2.6-kilobase insert expressing an immunoreactive protein of 28 kDa. DNA sequencing revealed that the insert contains three complete and two partial open reading frames (ORFs), designated 5' to 3' as ORFs A-E. The *peb1A* gene (ORF D) contains 780 bases encoding a 259-residue polypeptide having a calculated molecular mass of 28,181 Da. The peptide sequence starting at residue 27 matches that determined from amino-terminal sequencing of mature PEB1 from *C. jejuni*. The first 26 residues contain typical signal peptidase I and II cleavage sites. The deduced amino acid composition and pI of the recombinant mature protein are similar to those determined for purified PEB1. Gene bank searches indicated significant overall homology of *peb1A* and ORF C with operons for amino acid transport systems in other Gram-negative organisms. *peb1A* is homologous to the binding components of systems such as *glnH* (27.8%) and *hisJ* (28.9%), whereas ORF C has nearly 50% identity to *glnQ* and *hisP*. Thus, PEB1 could be involved both in binding to intestinal cells and in amino acid transport.

infections in volunteers (9) and monkeys (10) suggests that immunity to *C. jejuni* is induced by recurrent exposure; thus, it may be possible to develop a vaccine against *C. jejuni* enteritis. We have previously identified two antigenic proteins, PEB1 (28 kDa) and PEB3 (30 kDa), from *C. jejuni* that are commonly recognized by convalescent sera from patients with sporadic *C. jejuni* diarrhea (11) and that may be vaccine candidates.

PEB1 is conserved in all *C. jejuni* and *C. coli* isolates and is located on the surface of *C. jejuni* cells as identified by immunogold electron microscopy (12), indicating that it is a good target for the immune system (11). PEB1 (CBF1) plays a major role in adherence to HeLa cells, suggesting that it may be involved in *Campylobacter* colonization of the intestine (13). PEB1 is a lysine-rich basic (pI 8.5) protein without methionine at its amino terminus, suggesting that a leader peptide is cleaved during PEB1 maturation (11); the amino terminus of PEB1 has no significant homology to other known proteins (11). We thus undertook the molecular cloning and sequencing of the gene encoding PEB1 to determine its primary sequence, to understand its post-translational modification and intracellular transport, and because large-scale production of recombinant PEB1 in *Escherichia coli* will facilitate functional and immunological studies.

We report the cloning and sequencing of the PEB1 structural gene (which we name *peb1A*) from *C. jejuni* strain 81-176. The deduced amino acid sequence indicated that PEB1 has a cleaved 26-amino acid leader peptide; the complete molecule exhibits significant homology to *Enterobacteriaceae* glutamine-binding protein (*glnH*) (14), lysine/arginine/ornithine-binding protein (LAO) (15), and histidine-binding protein (*hisJ*) (16) and may be part of a homologous operon.

EXPERIMENTAL PROCEDURES

Enzymes and Chemicals—Restriction endonucleases, calf intestinal alkaline phosphatase, IPTG, and affinity-purified goat antibody to rabbit IgG conjugated with alkaline phosphatase were obtained from Boehringer Mannheim. Other important reagents were bacteriophage T4 DNA ligase (New England BioLabs, Inc.), nitrocellulose BA-85 (Schleicher & Schuell), 5-bromo-4-chloro-3-indolyl- β -D-galactoside (Sigma), and Sepharose CL-2B (Pharmacia LKB Biotechnology Inc.).

Bacterial Strains—*C. jejuni* strain 81-176 (ATCC 55026), used for PEB1 production and genomic DNA preparation, was isolated from an outbreak of *Campylobacter* diarrhea and has been demonstrated to be a virulent strain in monkeys (10) and in volunteers (9). *E. coli* strains Y1088, Y1089, and Y1090 have been described (17), and XL1-

* This work was supported in part by the United States Army Medical Research and Development Command and by the Medical Research Service of the Department of Veterans Affairs. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L13662.

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¹ The abbreviations used are: LAO, lysine/arginine/ornithine-binding protein; IPTG, isopropyl-1-thio- β -D-galactopyranoside; kb, kilobase(s); bp, base pair(s); PCR, polymerase chain reaction; ORF, open reading frame.

Blue (Stratagene, La Jolla, CA) was used for transformations.

Preparation of λ Bank with Insert DNA from *C. jejuni*—A genomic library was prepared from *C. jejuni* strain 81-176 as previously described (18). In brief, purified DNA (800 μ g) was sheared by sonication. DNA fragments of 1.0–10 kb were isolated and ligated to *Eco*RI linkers and then to dephosphorylated λ gt11 arms. The ligation mixture was added to a λ -packaging mixture (Gigapack, Stratagene) and titered on Y1088 cells.

Immunological Methods—Polyclonal antiserum to PEB1 purified from strain 81-176 was raised in a hyperimmunized rabbit as previously described (11). This serum recognizes heterologous PEB1 antigens in all *C. jejuni* and *C. coli* strains, but has essentially no reactivity against other *C. jejuni* or *C. coli* antigens. Immunological screening of the *C. jejuni* library was performed as previously described by Gotschlich *et al.* (19). SDS-polyacrylamide gel electrophoresis was performed on lysates, proteins were transferred to nitrocellulose membranes, and the blots were developed by an immunoenzymatic method (11).

Subcloning and Physical Mapping of Insert—For expression and mapping of the insert, the original clones in λ gt11 were digested with *Eco*RI, and the inserts were separated in low-melting-point agarose and ligated into the *Eco*RI site of phosphatase-treated pUC19. The ligation mixture was used to transform competent XL1-Blue *E. coli* cells, and carbenicillin-resistant transformants were isolated (20). Recombinant plasmids were purified (21) and digested with restriction endonucleases (22). Western blotting was performed with antiserum to PEB1 to identify expressed proteins.

DNA Analysis—The *C. jejuni* DNA insert in pUC19 (pPB119) was digested with exonuclease III to generate a series of nested deletion mutants (23) and also by bidirectional endonuclease deletion based on the restriction map. The nucleotide sequence of plasmid DNA was determined on both strands by the dideoxynucleotide chain termination reaction (24) and was analyzed with DNASTAR software to define open reading frames and restriction sites. The amino acid sequence of the deduced gene product was analyzed for hydrophobicity with the algorithm of Kyte and Doolittle (25) and for secondary structure with the algorithm of Garnier *et al.* (26). Nucleic acid and amino acid homologies to GenBank and EMBL data bases were examined with NUSCAN and PROSCAN programs using the method of Pearson and Lipman (27).

Amplification of *peb1A* Gene from *C. jejuni* Isolates Using Polymerase Chain Reaction (PCR)—Oligonucleotides were synthesized on a Milligen 7500 automated DNA synthesizer. PCR was performed with bacterial chromosomal DNA concentrations of 1 ng/ μ l and with primer annealing at 54 °C for 1 min, extension at 72 °C for 2 min, and denaturation at 94 °C for 1 min for 30 cycles. PCR products were electrophoresed on 1% agarose gel and purified (GeneClean, Bio101, Inc., La Jolla, CA) for restriction digestions.

Southern Blot Analysis—Restriction fragments generated by *Hind*III digestion of whole chromosomal DNA on 0.7% agarose gel were transferred to nylon membranes after denaturation and neutralization according to the method of Southern as described (22). Probe was labeled with [³²P]dATP by random priming, and hybridization was performed in 50% formamide buffer overnight at 42 °C (22).

RESULTS

Detection of Recombinant Bacteriophage Expressing *C. jejuni* PEB1 Protein—The λ gt11 bank of genomic DNA from *C. jejuni* strain 81-176 in Y1088 cells yielded 8.2×10^5 plaque-forming units with a 76.2% insertion rate. After amplification in Y1090 cells, the bank was screened with an *E. coli*-absorbed rabbit antiserum to purified PEB1 from strain 81-176 (11) to detect expression of recombinant clones bearing PEB1 antigens. Two positive plaques were detected from a 10-cm diameter Petri dish containing $\sim 10^6$ plaques. These two clones were plaque-purified and amplified on Y1090 cells to provide high titer stocks for further study.

Characterization of Recombinant Protein in λ gt11—To further characterize these two clones, we constructed lysogens of each in *E. coli* Y1089 cells. The lysogens grown with IPTG and analyzed by immunoblot with antiserum to PEB1 both produced an immunoreactive product migrating at ~ 28 kDa (Fig. 1), essentially identical in apparent molecular mass to

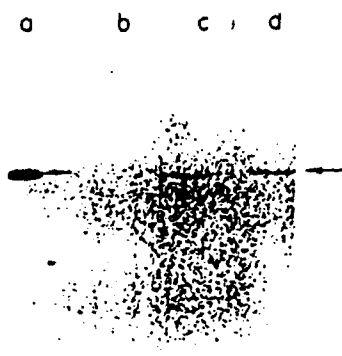


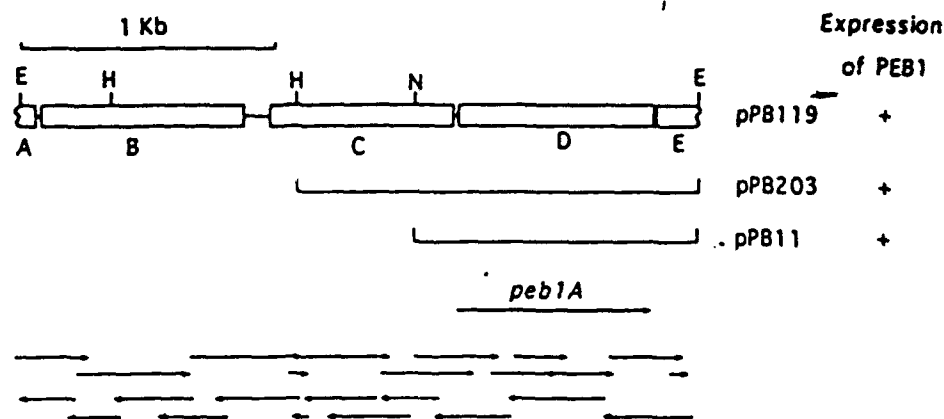
FIG. 1. Immunoblot of lysates of lysogenized *E. coli* Y1089 cells producing recombinant *C. jejuni* antigens. Lane a, cells of *C. jejuni* strain 81-176; lane b, cells of *E. coli* strain Y1089 containing λ gt11 without an insert; lane c, cells of lysogenic clone 1; lane d, cells of lysogenic clone 2. *E. coli* cells in lanes b–d were cultured overnight in the presence of 2 mM IPTG to induce expression of genes downstream of the *lacZ* promoter in λ gt11. A band migrating at ~ 28 kDa (indicated by arrow) was recognized by rabbit antiserum to purified PEB1 (1:10,000) (11) in clones 1 and 2, but not in the strain harboring λ gt11 alone.

PEB1 produced by *C. jejuni* strain 81-176. The DNA from each clone was purified, and the inserts were excised following *Eco*RI digestion and resolved by agarose gel electrophoresis. Each clone contained a single insert of 2.6 kb.

Characterization of *C. jejuni* Insert DNA—The 2.6-kb insert in clone 1 was subcloned into pUC19, and transformants were screened by immunoblot using antiserum to PEB1. The plasmids from two transformants producing immunoreactive molecules migrating at ~ 28 kDa had the insert in opposite orientations and were designated pPB119 (Fig. 2) and pPB219. Both strains expressed the molecule in the absence of IPTG, but IPTG enhanced expression in pPB119, indicating an effect mediated by the β -galactosidase promoter of the vector. *Hind*III and *Nco*I deletion mutants of pPB119 (pPB203 and pPB11) both expressed the full-length 28-kDa protein (Fig. 2).

Nucleotide Sequence of *peb1A*—The nucleotide sequence of the 2687-bp insert determined according to the strategy shown in Fig. 2 yielded three complete and two partial open reading frames (ORFs), which were designed 5' to 3' as ORFs A–E (Fig. 3). ORF A is a partial ORF encoding 21 amino acids, ending with TAA at positions 65–67. Between ORFs A and B, there are 15 nucleotides containing a putative (28) ribosomal binding site (AGGA, positions 72–75) 7 nucleotides upstream from the ATG codon initiating ORF B. No putative transcriptional terminator was found in this region, suggesting that ORFs A and B may be cotranscribed. ORF B is 795 nucleotides, encoding a 264-residue polypeptide, ending with TAA at positions 875–877. Following ORF B is a 128-nucleotide noncoding sequence containing an inverted repeat that could form a stem-loop structure ($\Delta G = -9.0$) (Fig. 3) (29). ORF C begins with an unusual start codon (TTG) at positions 1006–1008 (30, 31). A putative ribosomal binding site (AGGA) is located 6 nucleotides upstream from the TTG codon. There is a sequence (TAAAAT) resembling the -10 consensus sequence in *E. coli* (TATAAT) that is 35 bases upstream from the ribosomal binding site, and 20 nucleotides farther upstream, there is a sequence (TTGAAG) resembling the -35 consensus sequence in *E. coli* (TTGACA) (32). ORF C is 729 nucleotides, encoding a polypeptide of 242 amino acids, ending at positions 1732–1734 with TAA. ORF D follows ORF C after a 21-nucleotide noncoding region. A putative ribosomal binding site (AGGA) is located 6 nucleotides upstream from

FIG. 2. Restriction map of pPB119 and sequencing strategy for *peb1A* gene. Restriction sites are shown above the 2.6-kb insert (E, *EcoRI*; H, *HindIII*; N, *NcoI*). Three complete ORFs, B-D, and two partial ORFs, A and E, are indicated below the insert. The large arrow represents the direction of transcription of *peb1A*. pPB203 and pPB11 are deletion mutants of pPB119. Solid arrows represent sequences obtained from deletion mutants, and dotted arrows from primer sequencing.



the start codon (ATG) for ORF D at position 1756. ORF D (*peb1A*) is 780 nucleotides, terminated by TAA at positions 2533-2535, and encodes a polypeptide of 259 amino acids with a molecular mass of 28.18 kDa. One base downstream of ORF D, truncated ORF E begins; only the first 50 amino acids of this ORF can be deduced from the insert. Since no potential transcriptional terminators were found among ORFs C-E, it is possible that these ORFs are cotranscribed using a common promoter located upstream from ORF C. No ORF >300 nucleotides was found in the complementary strand.

Signal Sequence of PEB1—The amino-terminal amino acid sequence of mature native PEB1 (11) is identical to the deduced sequence from ORF D beginning at residue 27, indicating that mature PEB1 has a 26-residue cleaved signal sequence. Overall, the 26-residue signal peptide has a calculated molecular weight of 2742 and is similar in structure to a typical signal peptide (33, 34). Arg and Lys at positions 4 and 5, respectively, form its positively charged head; the next 9 residues form a hydrophobic core, followed by Gly, an α -helix breaker, 10 residues upstream from the cleavage site. A typical structure for signal peptidase I cleavage (33, 34) occurs between Ala²⁶ and Ala²⁷, followed by negatively charged Glu²⁸. Immediately following the cleavage site, 8 of 13 residues are polar. A second conserved signal peptidase-processing structure (Leu¹⁸-Gly¹⁶-Ala¹⁷-Cys¹⁹) homologous to signal peptidase II cleavage sites was located in which Cys is essential, Leu is highly conserved, and small amino acids between Leu and Cys such as Gly, Ala, Ser, and Val are preferred (35).

Amino Acid Composition and Codon Usage—Although ORF D (*peb1A*) encodes a deduced protein of 28.2 kDa, the deduced molecular mass of mature PEB1 (27th to 259th amino acid residue) is 25.5 kDa. The pI (8.51) of the deduced mature protein is nearly identical to the experimentally derived pI (8.5) of the mature protein (11). The amino acid composition determined for the mature protein from *C. jejuni* strain 81-176 (11) and that deduced for *peb1A* (27th to 259th residue) are in general agreement, with a few exceptions. The deduced mature protein contains 39 basic amino acid residues (33 Lys, 4 Arg, and 2 His) and 35 acidic residues (22 Asp and 13 Glu), indicating a net positive charge consistent with both the determined and predicted pI values. Cysteine is not found either in PEB1 or in the deduced mature *peb1A* gene product. Examination of the deduced amino acid sequence indicates relatively uniform distribution of positive and negative charges over the length of the molecule. Hydrophobic amino acids with no polar side chains represent 50% of the residues. The GC content of *peb1A* is 31.66%; A or T represents 88% (229/260) of the third-position nucleotides. This GC content and codon usage are consistent for *Campylobacter* DNA (18,

36-38), but as expected, are significantly different from *E. coli* (39).

Secondary Structure of *peb1A* Gene Product—Secondary structure calculations (26) for the deduced mature protein indicate that 78% of the 233 residues are in the α -helical conformation. The deduced signal peptide is entirely in the α -helical conformation. Using the method of Kyte and Doolittle (25), the only major hydrophobic region (Leu⁷-Ala²⁴) is located in the leader peptide (data not shown); the other minor hydrophobic regions are randomly distributed over the entire molecule, but none is sufficiently long for membrane spanning.

Homologies of PEB1 to Other Proteins—A search of the National Biomedical Research Foundation (PIR 21.0) showed 27.8% identity of the deduced *peb1A* product to *E. coli* glutamine-binding protein precursor (*glnH*) (14), 22.9% identity to *Salmonella typhimurium* LAO (15), and 28.9% identity to *S. typhimurium* histidine-binding protein (*hisJ*) (16, 40). Searches of a variety of regions of PEB1 show no significant homologies to other known proteins. The amino acid composition, molecular mass, and secondary structure are similar between PEB1 and *glnH*, *hisJ*, and LAO; however, PEB1 is significantly more basic than these other proteins. A pairwise alignment of the primary sequence did not show consecutive identical regions of >4 amino acid residues between PEB1 and *glnH* or LAO (Fig. 4). The relationship of PEB1 with amino acid-binding proteins was further confirmed by the homology of ORF C to other members of operons for glutamine and histidine transport systems. ORF C shares nearly 50% identity with the proteins *glnQ* and *hisR* (Fig. 5), which serve as membrane receptors for the binding proteins *glnH* and *hisJ*, respectively. Both *glnQ* and *hisP*, like ORF C, begin with uncommon start codons such as TTG and GTG (14, 15, 41). ORF E, the third member of the putative PEB1 operon, did not share significant homology with other known proteins in the limited sequence that was identified.

ORF B shares an overall 22-24% identity with a number of heat shock proteins belonging to the hsp70 (42-45) and hsp90 (46) families, such as the 78-kDa glucose-regulated protein of yeast, which facilitates the assembly of multimeric protein complexes inside the endoplasmic reticulum and binds immunoglobulin heavy chain (42, 45). Homology was found also between mouse brain microtubule-associated protein (47) and ORF A (45% identity) and ORF B (24% identity) (Fig. 6).

Conservation of *peb1A* Gene among *C. jejuni* Strains—We next sought to determine the conservation of *peb1A* among *Campylobacter* strains by Southern hybridization since PEB1 is apparently present in all *C. jejuni* strains examined, and a closely related molecule is found in *C. coli* (11). Initial analyses used as the probe a 702-bp PCR product from pPB119

C. jejuni PEB1, a Binding Component Homolog

ORF A	
His Leu Lys Pro Met Ser Leu Lys Glu Ile Lys Lys Glu Ile	14
G CAT TTA AAA CCT ATG AGC TTA AAA GAA ATT AAA AAA GAA ATT	43
ORF B	
Val Asn Phe Ile Asp Gln Asp Och	3
GTA AAT TTT ATT GAT CAG GAT TAA TAAAGGAAAATTGC ATG GAA AAA	91
S.D.	
Lys Ile Thr Pro Ser Glu Leu Glu Leu Asn Glu Phe Ile Lys Ile	18
AAA ATA ACT CCT AGC GAA TTG GAA CTT AAT GAA TTT ATA AAA ATT	136
Ile Asn Glu Met Ser Gly Ile Asp Leu Thr Asp Lys Lys-Asn Ile	33
ATC AAC GAA ATG AGT GGT ATT GAT TTA ACC GAT AAA AAA AAT ATA	181
Leu Ala Leu Lys Leu Asn Lys Phe Leu Glu Gly Thr Asn Thr Lys	48
CTA GCT TTA AAG TTG AAT AAA TTT CTT GAA GGA ACT AAT ACT AAA	226
Asn Phe Ser Glu Phe Leu Gly Lys Leu Lys Ser Asn Arg Gln Leu	63
AAT TTT TCC GAA TTT TTG GGA AAA TTA AAA AGC AAT AGA CAA CTT	271
Lys Gln Glu Thr Leu Asp Phe Val Thr Ile Gly Glu Thr Tyr Phe	78
AAA CAA GAA ACT TTA GAT TTT GTA ACC ATA GGT GAA ACT TAT TTT	316
Leu Arg Glu Leu Ala Gln Leu Lys Glu Ile Ile Tyr Tyr Ala Lys	93
TTA AGA GAA TTG GCT CAA TTG AAA GAA ATA ATT TAT TAT GCC AAA	361
Ser Leu Glu Lys Arg Val Asn Ile Leu Ser Ala Pro Cys Ser Ser	108
AGC TTA GAA AAG AGA GTA AAT ATC CTA AGC GCC CCT TGT TCA AGT	406
Gly Glu Glu Val Tyr Ser Leu Ala Leu Leu Ala Ala Gln Asn Phe	123
GGA GAA GAA GTA TAT TCT TTG GCA TTA TTG GCT GCA CAG AAT TTT	451
Ile Lys Asp Met Tyr Ile Leu Gly Val Asp Ile Asn Ser Ser Val	138
ATT AAA GAT ATG TAT ATT TTA GGC GTT GAT ATT AAT TCA ACT GTG	496
Ile Glu Lys Ala Lys Leu Gly Lys Tyr Gln Gly Arg Thr Leu Lys	153
ATT GAA AAA GCA AAA CTT GCA AAA TAT CAA GGA AGA ACT TTA CAG	541
Arg Leu Ser Glu Ser Glu Lys Arg Arg Phe Phe Leu Glu Ser Glu	168
CGA TTG AGC GAG AGT GAA AAA AGA AGC TTT TTT TTA GAA AGC GAA	586
Asp Lys Phe Tyr Thr Ile Asn Lys Asn Glu Leu Cys Thr Cys Lys	183
GAT AAA TTT TAT ACT ATT AAT AAA AAT GAG CTT TGT ACT TGT AAA	631
Phe Glu Leu Cys Asn Val Phe Glu Glu Lys Phe Ser Arg Leu Gly	198
TTT GAA CTT TGC AAT GTT TTT GAA GAA AAA TTT TCA AGA TTG GGA	676
Lys Phe Asp Ile Ile Ala Ser Arg Asn Met Ile Ile Tyr Phe Asp	213
AAA TTT GAT ATT ATA GCT TCT AGA AAT ATG ATT ATT TAT TTT GAT	721
His Glu Ser Lys Leu Lys Leu Met Glu Arg Phe His Arg Ile Leu	228
CAT GAA TCA AAA CTA AAA CTT ATG GAG AGG TTT CAT AGA ATT TTA	766
Asn Asp Lys Gly Arg Leu Tyr Val Gly Asn Ala Asp Leu Ile Pro	243
AAT GAT AAA GGA AGG CTT TAT GTT GGC AAT GCT GAT TTA ATT CCA	811
Glu Thr Ile Tyr Phe Lys Lys Ile Ser Leu Gln Glu Val Phe Thr	258
GAG ACT ATT TAT TTT AAA AAG ATT TCT CTC CAA GAG GTG TTT ACT	856
Met Lys Lys Tyr Lys Phe Och	264
ATG AAA AAG TAT AAA TTC TAA AAATTACTAAAAGTTACACTTTGGAAATTTA	908
-35 -10	
TTAGTAAAAATAAGTTACATTTTGAAGTAGTTTCTTTATTTAATGATAAAATAATTTTC	967
ORF C	
Met Ile Glu Leu Lys	5
AATTAATTTTATATTTAGCTAAAAATAAAGGAAAAAAC TTG ATT GAA TTA AAA	1020
S.D.	
Asn Val Asn Lys Tyr Tyr Gly Thr His His Val Leu Lys Ile Phe	20
AAT GTA AAC AAA TAC TAC GGA ACT CAT CAT GTT CTA AAG ATA TTT	1065
Asn Leu Ser Val Lys Glu Gly Glu Lys Leu Val Ile Ile Gly Pro	35
AAT CTT TCT GTT AAA GAA GGT GAG AAG CTT GTT ATT ATA GGT CCA	1110
Ser Gly Ser Gly Lys Ser Thr Thr Ile Arg Cys Met Asn Gly Leu	50
AGT GGA AGT GGA AAA AGT ACA ACT ATC CGT TGC ATG AAT GGG CTT	1155
Glu Glu Val Ser Ser Gly Glu Val Val Val Asn Asn Leu Val Leu	65
GAA GAA GTT AGT TCA GGA GAG GTC GTA GTT AAC AAT CTT GTT TTA	1200
Asn His Lys Asn Lys Ile Glu Ile Cys Arg Lys Tyr Cys Ala Met	80
AAT CAT AAA AAT AAA ATT GAA ATT TGC CGA AAA TAT TGT GCA ATG	1245
Val Phe Gln His Phe Asn Leu Tyr Pro His Met Thr Val Leu Gln	95
GTT TTT CAG CAT TTT AAT TTA TAT CCA CAT ATG ACC GTT TTG CAA	1290
Asn Leu Thr Leu Ala Pro Met Lys Leu Gln Lys Lys Ser Lys Lys	110
AAT TTG ACC TTA GCT CCA ATG AAA CTT CAA AAA AAA TCT AAA AAA	1335
Glu Ala Glu Glu Thr Ala Phe Lys Tyr Leu Lys Val Val Gly Leu	125
GAA GCT GAA GAA ACA GCT TTT AAG TAT TTA AAA CTT GTA GGT TTG	1380
Leu Asp Lys Ala Asn Val Tyr Pro Ala Thr Leu Ser Gly Gly Gln	140
CTG GAT AAA GCA AAT GTT TAT CCA GCA ACC CTT TCA GGT GGA CAA	1425

FIG. 3. Nucleotide and deduced amino acid sequences of 2687-bp pPB119 fragment containing *peb1A*. The DNA sequence was determined for both strands as described under "Experimental Procedures." The three-letter amino acid code and the termination codon TAA (*Och*) are indicated above each triplet nucleotide codon. Nucleotides for pPB119 and amino acids for each open reading frame are numbered on the right of each line. The ribosomal binding sites (Shine-Dalgarno (*S.D.*)) and the putative promoter are indicated, and the **boldface** portions of the DNA sequence represent inverted repeat sequences that may serve as a transcriptional terminator. The **boldface** amino acid sequence in ORF D was determined by amino-terminal sequencing of mature PEB1 from *C. jejuni* (11).

Gln Gln Arg Val Ala Ile Ala Arg Ser Leu Cys Thr Lys Lys Pro 155
 CAA CAA CGC GTT GCT ATA GCA AGA TCA CTT TGT ACT AAA AAA CCC 1470
 Tyr Ile Leu Phe Asp Glu Pro Thr Ser Ala Leu Asp Pro Glu Thr 170
 TAT ATT TTA TTT GAT GAA CCT ACT TCA GCC CTT GAT CCA GAA ACC 1515
 Ile Gln Glu Val Leu Asp Val Met Lys Glu Ile Ser His Gln Ser 185
 ATA CAA GAG GTT TTA CAT GTA ATG AAA GAA ATT TCA CAT CAA AGC 1560
 Asn Thr Thr Met Val Val Val Thr His Glu Met Gly Phe Ala Lys 200
 AAT ACT ACC ATG GTG GTT GTT ACA CAC GAA ATG GGT TTT GCA AAA 1605
 Glu Val Ala Asp Arg Ile Ile Phe Met Glu Asp Gly Ala Ile Val 215
 GAA GTA GCA GAT AGG ATT ATT TTT ATG CAA GAT GGT GCT ATT GTG 1650
 Glu Glu Asn Ile Pro Ser Glu Phe Phe Ser Asn Pro Lys Thr Glu 230
 GAA GAA AAT ATT CCT AGT GAA TTT TTC TCA AAT CCA AAA ATG GAA 1695
 Arg Ala Arg Leu Phe Leu Gly Lys Ile Leu Lys Asn Och 242
 AGA GCG CGA CTC TTT TTA GGG AAA ATT CTT AAA AAT TAA CCAAAAT 1741

ORF D

Met Val Phe Arg Lys Ser Leu Leu Lys Leu Ala 11
 TGAAAGGAGAAAAA ATG GTT TTT AGA AAA TCT TTG TTA AAG TTG GCA 1788
 S.D.

Val Phe Ala Leu Gly Ala Cys Val Ala Phe Ser Asn Ala Asn Ala 26
 GTT TTT GCT CTA GGT GCT TGT GTT CCA TTT AGC AAT GCT AAT GCA 1833

Ala Glu Gly Lys Leu Glu Ser Ile Lys Ser Lys Gly Gln Leu Ile 41
 GCA GAA GGT AAA CTT GAG TCT ATT AAA TCT AAA GCA CAA TTA ATA 1878

Val Gly Val Lys Asn Asp Val Pro His Tyr Ala Leu Leu Asp Gln 56
 GTT GGT GTT AAA AAT GAT GTT CCG CAT TAT GCT TTA CTT GAT CAA 1923

Ala Thr Gly Glu Ile Lys Gly Phe Glu Val Asp Val Ala Lys Leu 71
 GCA ACA GGT GAA ATT AAA GGT TTC CAA GTA GAT GTT GCC AAA TTG 1968

Leu Ala Lys Ser Ile Leu Gly Asp Asp Lys Lys Ile Lys Leu Val 86
 CTA GCT AAA AGT ATA TTG GGT GAT GAT AAA AAA ATA AAA CTA GTT 2013

Ala Val Asn Ala Lys Thr Arg Gly Pro Leu Leu Asp Asn Gly Ser 101
 GCA GTT AAT GCT AAA ACA GGC CCT TTG CTT GAT AAT GGT AGT 2058

Val Asp Ala Val Ile Ala Thr Phe Thr Ile Thr Pro Glu Arg Lys 116
 GTA GAT GCG GTC ATA GCA ACT TTT ACT ATT ACT CCA GAG AGA AAA 2103

Arg Ile Tyr Asn Phe Ser Glu Pro Tyr Tyr Gln Asp Ala Ile Gly 131
 AGA ATT TAT AAT TTC TCA GAG CCT TAT TAT CAA GAT GCT ATA GCG 2148

Leu Leu Val Leu Lys Glu Lys Lys Tyr Lys Ser Leu Ala Asp Met 146
 CTT TTG GTT TTA AAA GAA AAA AAA TAT AAA TCT TTA GCT GAT ATG 2193

Lys Gly Ala Asn Ile Gly Val Ala Gln Ala Ala Thr Thr Lys Lys 161
 AAA GGT GCA AAT ATT GGA GTG GCT CAA GCT GCA ACT ACA AAA AAA 2238

Ala Ile Gly Glu Ala Ala Lys Lys Ile Gly Ile Asp Val Lys Phe 176
 GCT ATA GGT GAA GCT GCT AAA AAA ATT GGC ATT GAT GTT AAA TTT 2283

Ser Glu Phe Pro Asp Tyr Pro Ser Ile Lys Ala Ala Leu Asp Ala 191
 AGT GAA TTT CCT GAT TAT CCA AGT ATA AAA GCT GCT TTA GAT GCT 2328

Lys Arg Val Asp Ala Phe Ser Val Asp Lys Ser Ile Leu Leu Gly 206
 AAA AGA GTT GAT GCG TTT TCT GTA GAC AAA TCA ATA TTG TTA GGT 2473

Tyr Val Asp Asp Lys Ser Glu Ile Leu Pro Asp Ser Phe Glu Pro 221
 TAT GTC GAT GAT AAA AGT GAA ATT TTG CCA GAT AGT TTT GAA CCA 2418

Gln Ser Tyr Gly Ile Val Thr Lys Lys Asp Asp Pro Ala Phe Ala 236
 CAA AGT TAT GGT ATT GTA ACC AAA AAA GAT GAT CCA GCT TTT GCA 2563

Lys Tyr Val Asp Asp Phe Val Lys Glu His Lys Asn Glu Ile Asp 251
 AAA TAT GTT GAT GAT TTT GTA AAA GAA CAT AAA AAT GAA ATT GAT 2508

ORF E

Ala Leu Ala Lys Lys Trp Gly Leu Och Met Asn Glu Ser Val 5
 GCT TTA GCG AAA AAA TGG GGT TTA TAA T ATG AAT GAA AGT GTA 2551

Gly Phe Val Glu His Leu Arg Gln Ile Leu Thr Ser Trp Gly Leu 20
 GGT TTT GTT GAA CAT TTA AGA CAA ATT CTT ACT TCT TGG GGT TTA 2596

Tyr Asn Glu Asn Ser Ile Ser Pro Phe Ala Val Trp Lys Phe Leu 35
 TAT GAT GAA AAT AGT ATA AGC CCT TTT GCG GTA TGG AAA TTT TTA 2641

Asp Ala Leu Asp Asn Lys Asp Ala Phe Ile Asn Gly Phe Ile Tyr 50
 GAT GCT TTG GAT AAT AAA GAT GCT TTT ATT AAT GGT TTT ATT TAT G 2687

FIG. 3—continued

(primers: 5'-GCAGAAGGTAACTTGAGTCTATT-3' (bp 1834-1857) and 5'-TTATAAACCCCATTTTTCGCTAA-3' (complementary to bp 2512-2535), corresponding to the start and end of the sequence encoding mature PEB1). Under high stringency conditions, this probe hybridized to a single 1.8-kb HindIII-digested chromosomal fragment from all three *C. jejuni* strains, but not to the other *Campylobacter* strains

examined (Fig. 7). When the same pair of primers was used in PCR analysis, a 702-bp PCR product was amplified from all three *C. jejuni* strains tested, as predicted, but from none of the *C. coli*, *Campylobacter lari*, or *Campylobacter fetus* strains tested (Fig. 8A). Restriction digestion of the *peb1A* PCR products amplified from each of the three *C. jejuni* strains demonstrated identical patterns (Fig. 8B), exactly as

[illegible]

FIG. 4. Pairwise alignment of PEB1 with two amino acid-binding proteins. Gap penalty = 4, deletion penalty = 5, and similarity is defined by PAM 250 matrix, as described (57). Indicated are identity (|) and conservative substitution for hydrophobicity and charge (:) and size (.) . For PEB1 *versus* glnH, there is 29.3% identity in a 233-amino acid overlap, and for PEB1 *versus* LAO, there is 23.7% identity in a 232-amino acid overlap.

expected from sequence analysis, indicating the high degree of conservation of the *pebIA* gene among *C. jejuni* strains.

DISCUSSION

PEB1, a surface-exposed conserved antigen in *C. jejuni* and *C. coli* that is commonly recognized by convalescent sera from infected patients and is involved in the binding of *C. jejuni* to eukaryotic cells, is possibly a vaccine candidate (11-13). In this study, we found that *peb1A*, the gene cloned using antibody to PEB1, is a homolog of the binding component in bacterial amino acid transport systems. Since a role for amino acid transport systems in bacterial pathogenesis has not been reported before, we sought to determine whether the properties of PEB1 and those of the recombinant *peb1A* product are similar. Although we have not yet performed studies to evaluate the role of the recombinant *peb1A* protein as a cell-binding factor, the following evidence establishes its identity to the native cell-binding factor PEB1. 1) *E. coli* transformed with pPB119 (containing *peb1A*) expressed a protein similar in electrophoretic migration, deduced isoelectric point, and amino acid composition to PEB1 from *C. jejuni*. The amino-terminal sequence determined by peptide analysis of mature PEB1 matches that deduced from the *peb1A* DNA sequence. A leader peptide was predictable (and observed) since PEB1 does not have an amino-terminal methionine and is an exported protein. The deduced molecular mass of the mature *peb1A* product is 25.5 kDa, slightly less than that determined by SDS-polyacrylamide gel electrophoresis (28 kDa), which could be due to the slower migration of a basic protein that has fewer net negative charges per residue. That the DNA sequence predicts Ala for the first position of the mature protein whereas amino-terminal sequencing showed Gly may be artifactual since the chromatographic behaviors of these 2 amino acids during peptide sequencing are similar. 2) We have purified the recombinant *peb1A* protein to homogeneity.

Antibody to PEB1 recognized the purified *peb1A* protein.^{2/3} We have specifically mutated the *peb1A* gene from wild-type *C. jejuni* strain 81-176 by allelic replacement. If *peb1A* encodes a protein other than PEB1, mutating *peb1A* should not affect expression of PEB1. Using immunoblotting with antibody to PEB1, we found the PEB1 band in strain 81-176, but not in the isogenic *peb1A*⁻ mutant.²

Protein sequence comparisons of PEB1 indicated homology to members in the LAO superfamily, including products of the *glnH*, LAO, and *hisJ* genes. These function as amino acid-binding proteins as part of periplasmic amino acid transport systems in Gram-negative bacteria. Within the superfamily, the *hisJ* and LAO products share the highest homology, reflecting their binding of basic amino acids (histidine for *hisJ* and arginine for LAO), and share a common cellular receptor, *hisP* (15). Similar to *glnH*, PEB1 exhibits an overall identity of ~25% to other superfamily members. However, other evidence strengthens the hypothesis that PEB1 belongs to this superfamily. Mature PEB1 and the other three proteins all are 25–26 kDa, and all contain a cleaved amino-terminal signal peptide, a high percentage of lysine, and conserved Lys-Lys sequences near the carboxyl terminus. The presence of a signal peptide and the absence of transmembrane domains in the mature protein are consistent with each being secreted beyond the cytoplasmic membrane. In the superfamily, PEB1 is most closely related to *glnH* in both sequence similarity and hydrophobicity distribution, especially between residues 58 and 163.

In several periplasmic binding protein-dependent transport systems (14, 41, 51-53), the binding protein and the membrane-associated components are each encoded in the same operon containing three or four structural genes. Genomic organization of ORFs A-E in pPB119 indicates that the 2.6-kb insert contains two partial operons separated by a noncoding region between ORFs B and C. The putative transcrip-

¹ Z. Pei and M. J. Blaser, unpublished data.

FIG. 5. Homology between ORF C and *glnQ* and *hisP*. On the consensus line, upper-case letters represent residues conserved in all three molecules at that position, and lower-case letters represent residues conserved in two of the three molecules.

ORF C	MIEIk-MV---nKyYGthhVLkIfnLavkeGEklVIIGPSGSGKSTtIR	45
<i>glnQ</i> (14)	MIEIK-MV---sKhfgptqVLhnIdLnlaqGEVvVIIIGPSGSGKSTILR	45
<i>hisP</i> (40)	MeEnKlNvidlhKryGeheVLkgvsLqanaGdVisIIIGsSGSGKSTfLR	49
consensus	mIe-KInvid--k--g---vIk-I-L---Gav-vIIIGpSGSGKST-lR	
ORF C	CmNgLE---E---vsSG---evvV---nnL-VlnhKnkieicRkycaMVf	82
<i>glnQ</i>	CINkLE---E---I-tSGdII--V---DGLkVndpKvdeRLiRqeaqMVf	83
<i>hisP</i>	CINfLEkpsEgsIvVnGqtInlVrdkDGqlkvadKnqRLiRtrltMVf	98
consensus	CiN-LEkpsEgsiv-sG---I--Vrdkdgl-v---Kn--rl-R---MVf	
ORF C	QhFnLyPHmTVLqNltlaPmklqkksKkEAEetAkyLkvVGLldkA-n	131
<i>glnQ</i>	QqFyLPHltALENVHfgPlrVrCanKeEAEklArelLAKVGLaERA-h	131
<i>hisP</i>	QhFnLvsHmTVLENVMeaPiqVIGIsKqEArerAvkyLAKVGIdERAqg	147
consensus	QhFnL-pHmTVLeNvm-aP--v-g-sK-EAee-A-kyLakVGL-erAq-	
ORF C	vYPatLSGGQQQRVAIARsLctKkpylLFDEPTSALDPETiqEVLdVMke	180
<i>glnQ</i>	hYPseLSGGQQQRVAIARALAVKPkmmLFDEPTSALDPELrhEVLkVM-Q	180
<i>hisP</i>	kYPvhLSGGQQQRVsIARALaePevlLFDEPTSALDPELvgElLriM-Q	196
consensus	-YP--LSGGQQQRVAIARaLa-kp---LFDEPTSALDPEL--EvL-vMkq	
ORF C	ishqsnTTHVvVTHEmGFAkeVAdRIIfmedGaIvEeniPseffsNPkte	230
<i>glnH</i>	dLAEEGmTHViVTHEiGFAekVAsRIIfidkGrIaEdGnPgqVLikNPpSq	230
<i>hisP</i>	qLAEEGkTMVvVTHEmGFARhVsthvIFlhqGkIeEeGaPeqLfgNPpSp	246
consensus	-laeeg-TMVvVTHEmGFA--Va-r-IF---G-I-Eeg-P--lf-NP-s-	
ORF C	RarIFLgkilkn	242
<i>glnQ</i>	RLqeFLqhvs	240
<i>hisP</i>	RLqrFLkgsIk	257
consensus	RIq-FL---lkn	

ORF A	HLKPHSLKEIKKEIVNFIDQD	21	ORF B	MEKK-ITPS	8
MAP1B(47)	PRKEEVKKEIKKEIKKEERKELKKEVKKETPLKDAKKEVKKEEKKEVKKE	722			
ORF B	EELNEFIKII--NEMSGIDLTDKKNILALKLNKFLEGTNTKNFSEFLGK	56			
MAP1B	EKEPKKEIKKISKDIKSTPQSDTKKPSALKPKVAKKEESTKKEPLAAGK	772			
ORF B	LKSNRQLK-----QETLDFVTIGETVFLRELAQLKEIIYYAKSLEKRVN	100			
MAP1B	LKDKGKVKVVIKKEGKTTEAAATAVGT--AATTAAVVAAAGIAAS--GPVK	818			
ORF B	ILSAPCS--SGEEVYSLALLAAQNFIKDHYI-LGVDI--NSSVIE-KAKL	144			
MAP1B	ELEAERSLMSSPE----DLTKDFEELKAEEDVAKDIKPKLELIEDEEKL	864			
ORF B	GKYQ-GRTLQRLSESEKRRFFLESEDKFYTINKNE-LCTCKFE-LCNVFE	191			
MAP1B	KETQGEAYVIQKETEVSKGSAESPDEGITTEGECEQTPPELEPV--	912			
ORF B	EKFSRLGKFDIIASRNHIIYF-----DHESKLKLMERFHRILNOKGRL	234			
MAP1B	EK---QGVDDIEKFEDGAGFEESSETGDYEEK-AETEEAEEPEDEGEDM	958			
ORF B	YVGNA DLIPETIYFKKISLQEVFTMKKYKF	264			
MAP1B	ASGSASKHSPT-EDDESAKAEADVHLKEKR	987			

FIG. 6. Homology between ORFs A and B and mouse brain microtubule-associated protein (MAP1B) (47). Parameters used were gap penalty = 1, gap size penalty = 0.05, and joining penalty = 20.

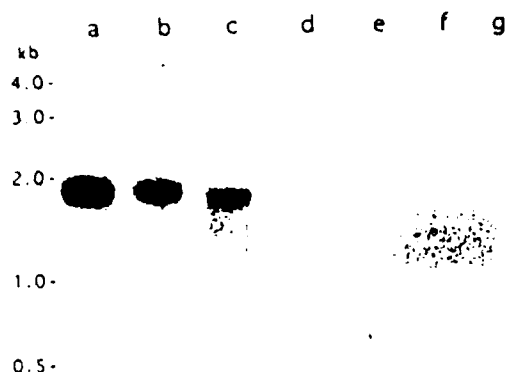


FIG. 7. Southern hybridization showing conservation of *peb1A* gene in *C. jejuni* chromosomal DNA digested with *Hind*III. A 702-bp PCR product corresponding to the DNA sequence of mature PEB1 was used as probe. Lane a-c, *C. jejuni* strains 81-176, 85-H, and 81-95, respectively; lanes d and e, *C. coli* strains D126 and D730, respectively; lanes f and g, *C. fetus* strains 23D and 84-91, respectively. Molecular size markers (in kilobases) are shown to the left.

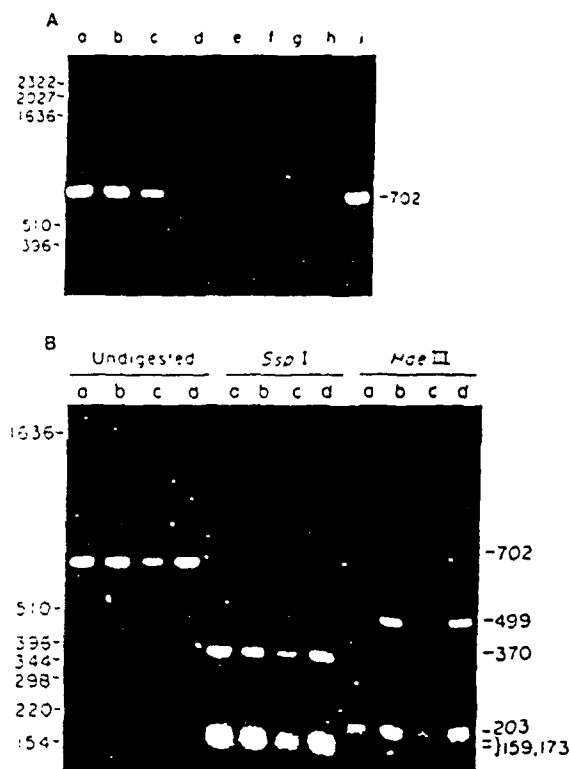


FIG. 8. A, PCR amplification of 702-bp *peb1A* fragment from *Campylobacter* strains. Lane a-c, *C. jejuni* strains 81-176, D1916, and 85AC, respectively; lanes d and e, *C. coli* strains D126 and D1035, respectively; lanes f and g, *C. lari* strains D110 and D67, respectively; lane h, *C. fetus* strain 23D; lane i, *E. coli* with pPB119. A 702-bp PCR-amplified product was found in all *C. jejuni* strains (arrow), but not in the other *Campylobacter* species. B, restriction pattern of 702-bp PCR products from *C. jejuni* strains. The 702-bp PCR products were undigested or were digested with *Ssp*I or *Hae*III. Strain 81-176 is shown in lanes a and d (undigested) and c (*Ssp*I-digested), strain D1916 in lanes b (undigested) and a and d (*Ssp*I-digested), and strain 85AC in lanes c (undigested), b (*Ssp*I-digested), and a (*Hae*III-digested). *Ssp*I cleaved the 702-bp PCR products from each strain into 370-, 173-, and 159-bp fragments, and *Hae*III cleaved the PCR products from each strain into 499- and 203-bp fragments, indicating that the *peb1A* gene is highly conserved in *C. jejuni*.

tional terminator in this region indicates a potential 3'-end of the operon containing ORFs A and B. The putative promoter just downstream indicates the 5'-end of the operon containing ORFs C, D (*peb1A*), and E. Support for this hypothesis includes the following. 1) The putative promoter is the only promoter found upstream from ORFs C-E, which could be responsible for *lacZ*-independent transcription of ORF D in pPB219; 2) there is no transcriptional terminator identified between ORFs C and D and between ORFs D and E; and 3) in *E. coli* and *S. typhimurium*, the genes homologous to ORFs C and D are randomly aligned and cotranscribed (14-16). A putative function of ORF E is unknown at present.

Although the homology between PEB1 and amino acid-binding proteins is significant, PEB1 is unique since all identified amino acid-binding proteins in bacterial transport systems are located in the periplasmic space, whereas PEB1 is exposed on the bacterial surface (12). Members of the LAO superfamily all contain a leader peptide processed by signal peptidase I, which enables these molecules to cross the cytoplasmic membrane. PEB1 also has such a processing site, as demonstrated by both the characteristics of the deduced PEB1 sequence and the evidence for cleavage at this site in *C. jejuni* (11). In addition, PEB1 contains a putative signal peptidase II cleavage site. Signal peptidase II processes lipoprotein precursors acylated on the free sulfhydryl group of cysteine by cleavage of the peptide bond at the amino-terminal side of cysteine. The acyl chains of the lipoprotein often anchor the polypeptide to membranes (54). Nearly all secreted proteins in bacteria have only one signal peptidase cleavage site processed by either signal peptidase I or II. However, the endoglucanase precursor of *Pseudomonas solanacearum* has a signal sequence of 45 residues with two processing sites (55). The endoglucanase is modified by fatty acylation at Cys²⁰, cleaved by signal peptidase II, and exported across the inner membrane. The lipoprotein intermediate is then cleaved at the signal peptidase I-like site (Ala⁴⁵-Ala⁴⁶) during export across the outer membrane. Since PEB1 also contains two signal peptidase-processing sites, the location of PEB1 on the bacterial surface suggests that *C. jejuni* could utilize similar mechanisms to export this protein across both the cytoplasmic and outer membranes. If this hypothesis is true, we would expect that mature PEB1 is not a lipoprotein since the final cleavage at Ala²⁷ eliminates any amino acid residues located between residues 1 and 26, including the putatively acylated Cys²⁰. This hypothesis was supported by the evidence that mature PEB1 begins at Ala²⁷ and that amino-terminal sequencing was not blocked by lipid (11).

We speculate that the major cell-binding factor PEB1 may have a common evolutionary origin with periplasmic amino acid-binding proteins, from which PEB1 gains the binding capacity. Since the genome of *C. jejuni* is only half the size of that of *E. coli* or *Salmonella* (56), *C. jejuni* may use particular proteins for multiple purposes. Two-step cleavage of the PEB1 leader peptide may distinguish it from these amino acid-binding proteins and make it accessible to the bacterial surface to perform cell binding functions as well.

The codon usage for PEB1 shows strong third-position AT preference; consequently, Arg, Asn, Cys, Gln, His, and Tyr are single-codon amino acids, which has been previously observed for *C. jejuni* serine hydroxymethyltransferase (*glyA*) (48). Knowledge of this phenomenon will be helpful in designing oligonucleotide probes to clone other *C. jejuni* proteins for which antibody probes are not available.

ORF C begins with TTG, which although not common, has been previously identified (30, 31, 41, 49, 50). The ORF C and *glnQ* and *hisP* gene products are highly homologous, and their



ORFs also begin with uncommon initiation codons (14, 15). Experimental replacement of TTG with ATG led to a 2.5–3.7-fold increase in protein translation (49, 50). The low translation efficiency when TTG is the initiating codon suggests a possible conserved mechanism for control of expression of this family of homologous proteins.

Although both *C. jejuni* and *C. coli* strains contain PEB1 homologs (11), using primers corresponding to the amino and carboxyl termini of mature PEB1, we PCR-amplified the expected fragment from *C. jejuni* strains, but not from *C. coli* strains. Amino-terminal differences in the *C. jejuni* and *C. coli* PEB1 homologs (11) correlate with the failure of both amplification and hybridization of the *peb1A* gene with *C. coli* strains. In contrast to *C. coli* strains, the primary sequence of *peb1A* must be highly conserved among *C. jejuni* strains, as shown by the conserved PCR product restriction profiles, again indicating its importance to the organism.

Acknowledgment—We thank Charlene Oakley for typing this manuscript.

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